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BILAYER INTEGRITY-MAINTAINING ION PAIR AMPHIPHILES: PAMAM DENDRIMER-INDUCED MORPHOLOGICAL ADAPTATION OF HYBRID CATIONIC VESICLES

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ABSTRACT

Nanoparticles of various types have been used as drug delivery vehicles in medical therapies. **T**he main aim of the study is Bilayer integrity-maintaining ion pair amphiphiles: PAMAM dendrimer-induced morphological adaptation of hybrid cationic vesicles.The traditional thin film approach was used to create hybrid cationic vesicles with varying compositions. To make cationic vesicles, the following ratios of IPA (10:0, 9:1, and 7:3; M/M) and cholesterol (30 mol%) were used: 35,36 SLC: IPA (10:0, 9:1, and 7:3; M/M).To learn more about the morphological adaptability of PAMAM-succinamate dendrimers with a 1,4-diaminobutane core, generation 5, researchers studied their interaction with hybrid cationic vesicles made up of SLC, IPA, and DHADB.

Keywords: Nanoparticles, morphological, adaptability, adaptation, traditional

1. INTRODUCTION

Nanoparticles of various types have been used as drug delivery vehicles in medical therapies. Drug delivery vehicles have been used for a long time, including micro emulsion, solid lipid nanoparticle (SLN), vesicle, dendrimer, etc. Yet, a significant obstacle persists since the mechanism of interaction between nanoparticles and cell or plasma membrane is still poorly known. To investigate this interaction, one has to strategically create nanoparticles with the right kind of components, in the right kind of quantities, and with the right kind of shape and size. It should be biodegradable, have minimal cytotoxicity, and efficiently deliver the medicine, gene, or bioactive chemicals to the intended tissue while withstanding the body's natural conditions.

The interesting hydrophiliclipophilic nature and adaptable architecture of vesicles made mostly of phospholipids have made them a popular tool for decades. To foretell how a medication or other macromolecule, like dendrimers, would interact with a lipid bilayer, it is helpful to first understand how lipid bilayers function in biological cells. Yet, constraints such as stability have

prompted scientists to create a new category of molecule that may circumvent these problems. There has been a lot of research into using polyamidoamine (PAMAM) dendrimers as vaccine carriers, medication delivery vehicles, gene delivery vehicles, etc. Dendrimers' structure and synthesis have both been well described.

2. LITERATURE REVIEW

Xiao, Qi &Yadavalli, Srujana& Zhang(2016) Glycans, receptors, and adhesion molecules, all found on the cell surface, play a role in the intricate regulation of cell sociology. Evidence for the viability of merging chemical and biological surface design is achieved by fusing chemically programmable (glyco)dendrimersomes with bacterial membrane vesicles to create cell-like hybrids. Dissecting the function of individual entities in complex networks using such cell-like hybrids with tailor-made combinations of surface epitopes and active receptors will likely find use in enabling innovative biomedical applications.

Liu, Karen & Yeo, Yoon (2013) Dendrimers based on polyamidoamine (PAMAM) have been studied extensively for their potential as drug and imaging agent carriers. Due to their cationic charges, amine-terminated PAMAM dendrimers pose a danger of opsonization and cytotoxicity, limiting its usage in systemic applications. The use of polymers capable of lowering the charges on the PAMAM dendrimer surface as a barrier should help alleviate these unintended consequences. Yet, this protection may also hinder PAMAM dendrimers' interaction with target cells, leading to decreased cellular uptake and overall delivery system performance. This is why we suggest using the novel chitosan derivative zwitterionic chitosan (ZWC), which has a pH-sensitive charge profile, as an alternative biomaterial to adjust the cationic surface of PAMAM dendrimers. At pH 7.4, fluorescence spectroscopy and transmission electron microscopy showed that ZWC and PAMAM dendrimers formed a stable electrostatic complex in which ZWC coated the PAMAM dendrimer surface. ZWC coating prevented the PAMAM dendrimers from hemolyzing red blood cells and killing fibroblast cells. Confocal imaging revealed that PAMAM dendrimers were able to penetrate cells despite ZWC's protective action at low pH, as the complex fragmented due to the charge conversion of ZWC. Our findings show that ZWC may coat PAMAM dendrimers' surfaces in a pH-dependent way, making them more effective drug carriers in solid tumours with an acidifying milieu.

Nyitrai, Gabriella &Keszthelyi(2013) Dendrimers made from polyamidoamine (PAMAM) are known to interact with cell membranes because they are highly charged hyperbranched polymers that resemble proteins. Monitoring the effect of PAMAM generation five (G5) dendrimer on the membrane permeability of living neuronal cells and then investigating the underlying structural changes with infrared-visible sum frequency vibrational spectroscopy (SVFS), small angle X-ray scattering (SAXS), and transmission electron microscopy (TEM) allowed us to shed light on the

mechanisms of dendrimer-membrane interaction (TEM). In low-[Na(+)] but not low-[Ca(2+)] environments, the permeability increase caused by G5 dendrimers was prevented, indicating the establishment of specialised Na(+) permeability channels. Results from SFVS experiments on DPPG-DPPC bilayers supported by silica revealed that the membrane trans-polarized in a G5-specific manner. Polar interactions between the G5 terminal amino groups and the anionic head groups of DPPC were shown by SAXS data and freeze-fracture TEM imaging of self-organized DPPC vesicle systems to be responsible for the rupture of DPPC vesicle layers upon addition of G5. We postulate a nanoscale process whereby G5 integrates into the membrane through numerous polar contacts that break the nearby membrane bilayer and construct a novel hydrophilic Na(+) ion permeable channel around the dendrimer. We also investigated the possibility that these synthetic Na(+) channels may be used as antibiotics. We demonstrated that G5 rapidly halts the growth of resistant bacterial strains at concentrations as low as 10 g/ml, without affecting the survival of red blood cells, providing hope for the creation of next-generation antibiotics that are effective against resistant strains.

Hong, Seungpyo& Rattan, Rahul &Majoros(2009) We generated dendrimers of generation 7 (G7) poly(amidoamine) (PAMAM) containing amine, acetamide, and carboxylate end groups to study polymer/cell membrane interactions in vitro. Since higher-generation dendrimers, including G7 PAMAM dendrimers, are more effective at forming nanoscale holes in supported lipid bilayers and permeabilizing cell plasma membranes, they were employed in this research. (1)H NMR, UV/vis spectroscopy, GPC, HPLC, and CE were used to characterise dendrimer-based conjugates. KB, Rat2, and C6 cells were shown to internalise a 200 nM concentration of positively charged amine-terminated G7 dendrimers (G7-NH(2)). On the other hand, G7 dendrimers ended with carboxylate (G7-COOH) or acetamide (G7-Ac) did not connect with the plasma membrane or get internalised under the same circumstances. Further elucidating the processes of dendrimer internalisation into cells, a series of in vitro investigations were carried out using the endocytic markers cholera toxin subunit B (CTB), transferrin, and GM(1)-pyrene. Dendrimers of G7-NH(2) were found colocalizing with CTB; however, C6 cell tests showed that internalisation of G7-NH(2) did not need ganglioside GM(1). Direct contact between the two species was thus considered to be the cause of the G7/CTB colocalization. Cell survival (as measured by XTT) and membrane permeability (measured by lactate dehydrogenase, or LDH) tests were unaffected by the presence of GM(1) in the membrane.

Gardikis, Konstantinos&Hatziantoniou(2006) The current research aimed to combine dendrimer and liposomal technologies to build novel controlled released systems for bioactive chemicals by investigating the interaction between PAMAM generation 4 (G4) dendrimer and model lipid membranes (DPPC). By using thermal analysis and Raman spectroscopy, we were able to determine the precise position of the PAMAM G4 (polyamidoamines) dendrimer inside the DPPC lipid bilayer and evaluate the thermodynamic alterations it generated. The DSC

thermograms showed that 5% PAMAM G4 may be added to the DPPC membrane without compromising its stability. Absorption at 715 cm1 revealed a robust contact between PAMAM G4 and the polar head group of phospholipid, whereas Raman intensity ratios I 2935/2880 and I 1090/1130 cm1 revealed the degree of flexibility of the lipid bilayer. PAMAM G4 dendrimers significantly interact with both the lipophilic component and the polar head group of the phospholipids, and the findings demonstrated that their incorporation into DPPC bilayers increases membrane fluidity as a function of concentration. Furthermore, these findings may provide justification for the propensity of dendrimers to disrupt biological membranes, given the limited understanding of how dendrimers interact with lipidic membranes at present.

3. METHODOLOGY

3.1 Methods

3.1.1 Preparation and Isolation of Ion Pair Amphiphile (IPA)

Hexadecyltrimethylammonium-dodecylsulfate (HTMA+ - DS-) was made by combining a stoichiometric quantity of two oppositely charged aqueous surfactant solutions, as stated. The IPA was characterised by 1 H-NMR, XRD, and FTIR.

3.1.2 Preparation of Vesicles

The traditional thin film approach was used to create hybrid cationic vesicles with varying compositions. To make cationic vesicles, the following SLC was used: IPA (10:0, 9:1, and 7:3; M/M) with 30 mol% cholesterol and 5 mol% DHDAB. A rotary evaporator was used to remove the solvent from a combination of SLC, IPA, DHDAB, and cholesterol mixed in a 3:1 chloroform/methanol mixture in a round-bottom flask. To get rid of the last of the solvent, the flask was put under vacuum at ambient temperature for a whole night. To rehydrate the film, it was placed in double-distilled water at 70 o C for 1 hour, which is above the chain melting point of all the lipidic components. Four to five freeze-thaw and sonication cycles were used to finally get a uniform dispersion.

4. RESULTS

4.1 Combined Dynamic Light Scattering (DLS) and Turbidity Measurement

The stability of vesicles is significantly affected by their hydrodynamic size (dh). Panel A of Figure 4.1 shows how the dh of vesicles would alter if different concentrations of G5-SA dendrimer were added. The highest adsorption of dendrimer to the vesicle surface occurred at a

concentration of 0.01 M G5-SA, and subsequent growth in vesicle size began at this point for all samples.

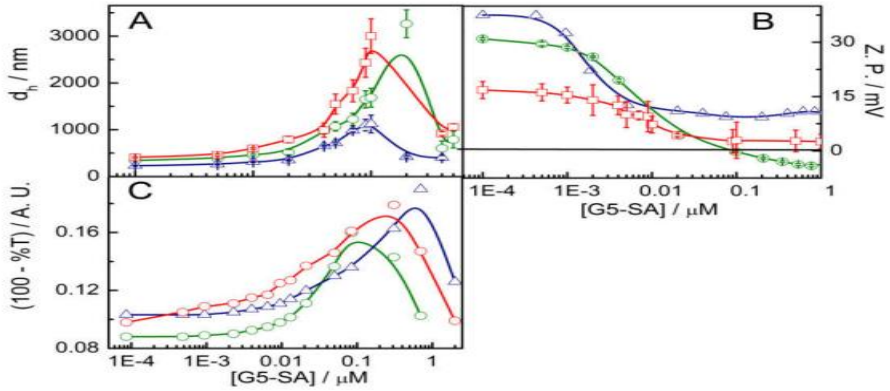

Figure 4.1. Variation of hydrodynamic size (dh, panel A), zeta potential (Z. P., panel B) and turbidity (T, panel C) with G5-SA concentration at 25 ºC. Vesicles (HCV1, ○; HCV2, Δ and HCV3, □.) with 0.1 mM lipid/surfactant concentration were used.

The results point to the dispersion-flocculation-dispersion steps being experienced by the vesicle dispersions. When the concentration of G5-SA is less than 0.01 M, it behaves as a weak electrolyte solution (Lipid/Dendrimer ratio = 107:1), failing to cause significant contact with the vesicles and hence resulting in only small changes in their sizes. At this concentration, the cationic vesicles and negatively charged dendrimer may interact electrostatically, leading to the commencement of adsorption of dendrimer to the vesicle surface. Vesicles of the HCV3 and HCV1 types saw more size augmentation than those of the HCV2 type. The hydrophobic action of 10 mol% IPA in the bilayer causes HCV2 to be smaller than the other two. In contrast, HCV1 grows when IPA is absent and HCV3 expands when IPA concentrations are over 30 mol%. As IPA was the sole dynamic component in the bilayer, it was hypothesised that it serves a critical role in preserving bilayer shape.

4.2 Transmission Electron Microscopic Studies (TEM)

The dendriosome, a combination of dendrimers and vesicles, has been hypothesised to arise in the postmaximal zone. TEM investigations are a worthy endeavour for elucidating the morphological modifications to vesicle structure caused by dendrimer. Changes in vesicle morphology as a result of exposure to dendrimer at low, intermediate, and high concentrations are shown in Figure 4.2, which was generated using electron microscopic examination.

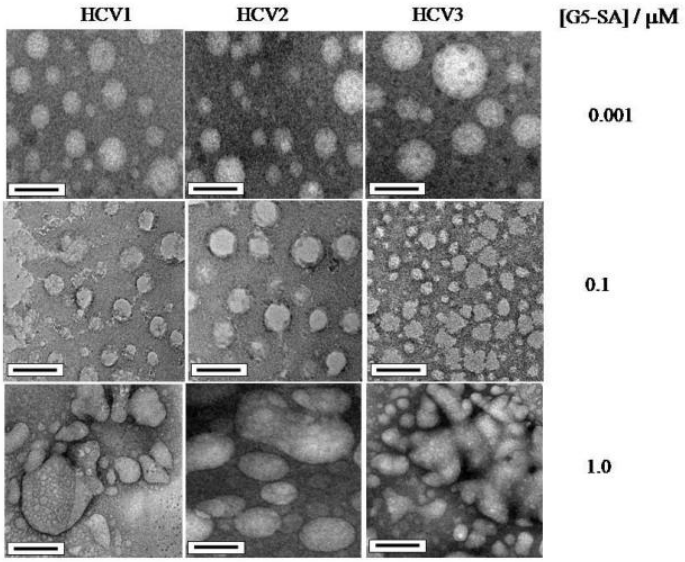

Figure 4.2Changes in vesicle morphology when G5-SA dendrimer concentration was varied.

4.3 Atomic Force Microscopic (AFM) Studies

Due of its spherical form, vesicle surfaces change dramatically following dendrimer contact, making TEM analysis insufficient for understanding the process. Hence, an AFM research would be excellent, since it would allow for a molecular-level investigation of the bilayer surface as a flat bilayer. Dendrimer-induced modifications to the surface characteristics of a bilayer may also be investigated. Hydrophobic effect that tends to increase impact between bilayer tail and hydrophobic core of dendrimers, leading to bilayer breakdown, is aided by their adsorption onto the bilayer surface through electrostatic contact.

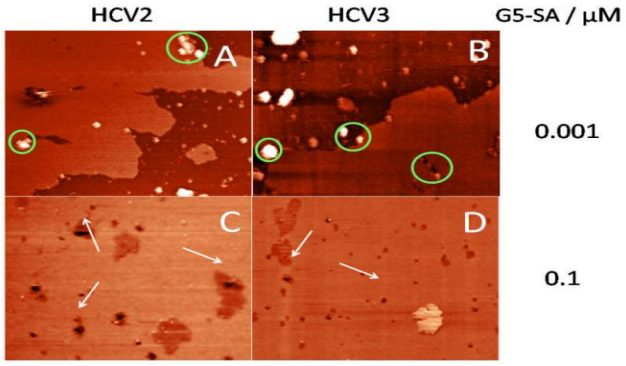

Figure 4.3 AFM micrographs of solid supported bilayers of HCV2 (10 mol% IPA) and HCV3 (30 mol% IPA) at 0.001(panel A and B) and 0.1 µM (panel C and D) G5-SA. Images were taken 2 h after the mixing

4.4 Vesicle Disruption and Subsequent Interfacial Adsorption Studies

The Z. P. measurement confirmed that the electrostatic interaction between the vesicles and dendrimer takes place mostly at the vesicle surface. Dendrimers induced vesicle bilayer breakdown was the intended subsequent consequence of this investigation. The creation of monolayers from bilayers at the water-air interface and their individual characteristics were the subject of substantial research. It is well established that vesicles in the bulk subphase may undergo a monolayer transformation at the water-air contact by undergoing a bilayer breakdown. Hence, the -t measurement may be used to investigate these processes using a Langmuir-Blodget surface balance. At the water-air interface, we validated the emergence of a monolayer and tracked its expansion as a function of both surface pressure () and time. The surface pressure fluctuations over time, as seen in Figure 4.4, are a byproduct of the creation of a monolayer at the water-air interface.

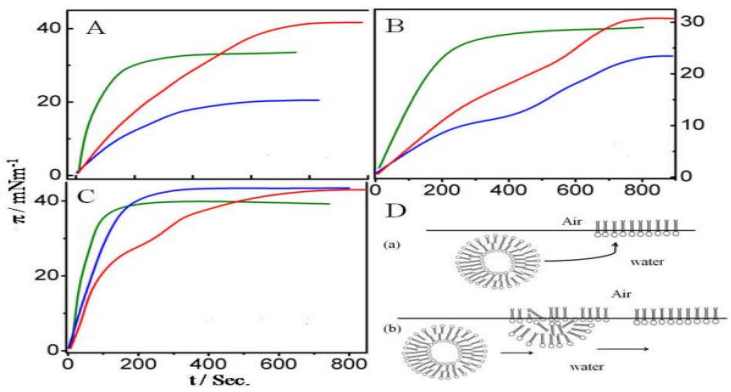

Figure 4.4: Breakdown of vesicles at the water-air interface leads to the formation of an interracially adsorbed monolayer, both without and with dendrimer. Changes in surface pressure (π) are shown in sequential panels. Panels A and B contain no dendrimers; Panel C contains 0.001 M; and Panel A contains 0.1 M. Line in green represents HCV1, line in blue represents HCV2, and line in red represents HCV3. The adsorption of a monolayer from hybrid cationic vesicles at the water-air interface may occur in two stages, as shown in Panel D.

To a greater or lesser degree, vesicles tend to break apart at the point when the aggregate comprising amphiphiles exit the bilayer area and preferentially orient themselves towards the water-air interface. With time, an increasing number of amphiphiles migrate across the water-air

interface after being released from the bilayer. When the interfacial coating becomes saturated, the surface pressure first rises and then reaches a plateau.

Once again, the function of IPA was remarkable; at 10 mol% IPA, the vesicles (HCV2) retain their unique character. With HCV3, however, the IPA head group takes centre stage, causing a perturbation in the vesicle surface area that helps bring about equilibrium pressures of 31 mN/m. The isotherms of HCV2 and HCV3 showed evidence of platue production during monolayer development; this was not the case for HCV1 (panel B). As HCV1 was the sole parameter whose percentage changed in the vesicles, the striking character of the isotherm must be due to the presence of IPA. Figure 4 panel D depicts two distinct routes by which a monolayer may be formed from a vesicle bulk. In (a), a monolayer was formed directly at the water-air interface, whereas in (b), a gaseous monolayer was formed with the help of additional lipidic aggregates to create a temporary monolayer island. After then, monolayers are adsorbed onto the monolayer island until it is completely saturated.

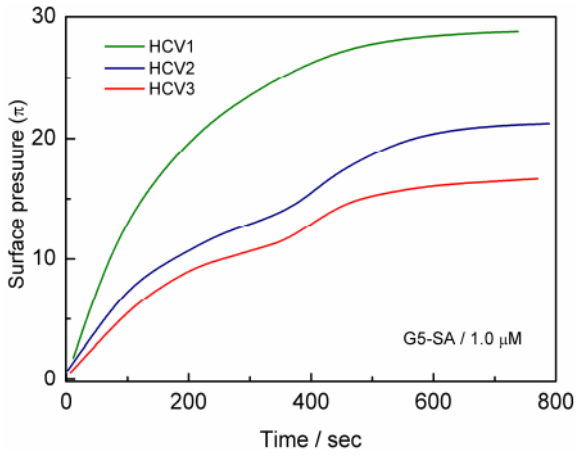

Figure 4.5Dendrimer/vesicle surface pressure evolution over time. The graphic identified the kind of vesicle and the dendrimer concentration.

Plan (b) is required from the platue, and it applies to both HCV2 (10 mo% IPA) and HCV3 (30 mo% IPA). Nevertheless, vesicle HCV1 (0 mol% IPA) does not exhibit the same behaviour and instead forms a monolayer directly (scheme a). It follows that IPA must provide structural support in order to retain the aggregates, which in turn promotes the creation of an initial monolayer island and a subsequent saturated monolayer at the interface.

Panel C of Figure 4.4 shows adsorbed monolayer development at 0.1 M G5-SA concentration, where the lipid to dendrimer stoichiometric ratio was 103:1. When comparing the formulations in panels A and B, the dendrimer formulation results in a relatively fast equilibrium pressure,

suggesting that dendrimer produces rapid membrane rupture. A gradual breakdown was seen at larger dendrimers concentrations, as shown in Figure 4.5. Evidence shows that G5-SA dendrimer has a high affinity for binding with hybrid membranes at high concentration and low surface pressure, two characteristics shared by cells undergoing fast miotic division such as cancer cells.

5. CONCLUSION

To learn more about the morphological adaptability of PAMAM-succinamate dendrimers with a 1,4-diaminobutane core, generation 5, researchers studied their interaction with hybrid cationic vesicles made up of SLC, IPA, and DHADB. When a dendrimers solution was added to vesicles, it had a clear effect on the shape of the vesicles. Dendrimers cause cationic vesicles to disperse, flocculate, and disperse, according to a systematic analysis of morphologies. Ionic bonding causes dendrimers to alter shape, as seen by a decrease in Z. P. with increasing concentration. Dendrimer effects were further investigated by analysing the kinetics of vesicle disintegration, where monolayer formation was seen after the breakdown of bilayers. Disintegration may be affected by dendrimers, according to research. The disintegration kinetics slowed down at low dendrimer concentrations but picked up again when the dendrimer concentration was increased. Adsorption of modest amounts of dendrimers and the process of bilayer breakdown caused by big amounts of dendrimers are both confirmed by AFM micrographs. Dendrimer incorporation into HCV2 and HCV3 vesicles did not result in the formation of a distinct phase or domain, proving the new ability of IPA to maintain bilayer integrity. DSC testing revealed the temperature at which the phase transition occurred; surface interactions between dendrimers at low concentration predominated. While we have seen bilayers that don't melt at the chin due to the presence of dendrimers at greater concentrations, we have also seen them be disrupted by these dendrimers. A propensity for dendrimer to interact with the gel phase was seen at this dosage. By taking into account their interaction on a molecular scale, the effects of negatively charged dendrimers on cationic vesicles and their morphological adaptation may be more fully understood by collaborative physicochemical studies. The area of drug delivery may benefit from the encapsulation of medicinal molecules within dendrimer/vesicle aggregates.

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